## SEED GERMINATION IN SIDALCEA NELSONIANA (MALVACEAE)

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<u>Sidalcea nelsoniana</u> Piper (Malvaceae) is an endemic species of the Willamette Valley and Coast Range of western Oregon (Halse and Glad, 1986) and is a Category 2 candidate for possible federal listing as threatened (U.S. Fish and Wildlife Service, 1985). The discovery of a population of <u>Sidalcea nelsoniana</u> within the area of a proposed water supply reservoir initiated a study program funded by the City of McMinnville, Water and Light Department and managed by CH2M Hill, Inc. to determine if experimental populations of this species could be successfully established in other suitable locations. One study in this program was to determine if large numbers of <u>Sidalcea nelsoniana</u> could be propagated from seed. This paper describes the germination studies.

Seed germination in the Malvaceae has not been closely studied except for economically important species. In the Gossypieae, Fryxell (1981) states that the seed coat is relatively indurate, is impregnated with lignins and tannins, is impervious to moisture, and has a degree of dormancy. This dormancy is broken after weathering or after the seed coat is physically damaged. Seeds of Alcea rosea L., hollyhock, will germinate after 14 to 21 days when kept in moist soil at 18 to 24°C; seeds of Abelmoschus esculentus (L.) Moench, okra, germinated after 4 to 14 days when kept in moist soil at 20 to 30°C (U.S. Dept. Agric., 1961). Within the genus Sidalcea, Sullivan and Daley (1981) report that the seeds of Sidalcea malvaeflora (DC.) Gray will germinate after being soaked in hot or warm water for 6 hours. The seeds of Sidalcea nelsoniana were reported by J. Kierstead (personal communication, 1985) to germinate if the seed coat was punctured or broken. With this information, a study was designed to develop large scale germination techniques for Sidalcea nelsoniana.

MATERIALS AND METHODS - The fruit of <u>Sidalcea nelsoniana</u> is a schizocarp, each carpel containing one seed. The carpel and seed dehisce as a single unit. The carpel wall, when mature, is dry and membranous. The seeds are arcuate, with the length averaging 2 mm and the width 1.5 mm. When fully mature, the seed coat is hard and shiny brown; when immature, the seed coat is softer and a dull greenish color.

Seeds of <u>Sidalcea nelsoniana</u> were collected during the late summer and early fall of 1985 from throughout the range of the species. Entire, dried inflorescences were picked and then stored in a cold room at 2°C until December 1985 and January 1986. At the time of collection the carpels were still partially enclosed by the persistent dry calyx.

Prior to germination, the following techniques were implemented for fruit preparation, seed scarification, and water and light exposure. The fruits were gently rubbed between two pieces of fine sandpaper to remove the carpel wall and the calyx. The papery calyx and carpel disintegrated, leaving the hard seeds intact. This process did not damage the seeds, other than leaving fine scratches on the seed coat. A block of slate drilled with holes about 3 mm in diameter and 2 mm in depth was used to hold the seeds. One seed was placed in each hole and the hard seed coat was ruptured with a finetipped probe. The scarification method used was to pry off a fairly large piece of the seed coat. The seed coat on a mature seed is quite hard and will crack off in pieces if pressure is applied in the appropriate manner. It was easiest to remove a piece of the seed coat from the dorsal surface of the seed. This process was carried out under 10X magnification.

The scarified seeds were then placed on moist filter paper in a petri dish where the embryos imbibed water and swelled, frequently escaping entirely from the seed coat (which remained intact). This usually occurred within 24 hours. If the embryo did not escape from the seed coat within the next 24 hours, it was squeezed out manually. The seed's proximal and distal ends were gently squeezed with forceps to expell the embryo. Germination occurred within 1 to 4 days of the breaking of the seed coat.

Once embryos had escaped from their seed coats they were allowed to remain on the moist filter paper for at least 24 hours. During that time rapid growth was observed. The cotyledons expanded, turned green, and frequently opened. The root increased in length and developed root hairs. Embryos were transferred to peat pots filled with potting soil when the cotyledons turned green or when root hairs developed, a process which took 2 to 7 days.

The effect of light on germination was also studied. Thirty seeds with unbroken seed coats were kept in the dark and another 30 seeds were kept in the light, all on moist filter paper in petri dishes. This treatment was repeated using the same number of scarified seeds. The majority, over 3000, of the scarified seeds were germinated in the light.

Germinating seeds sometimes became moldy. Surface sterilization with a weak bleach solution for 1 to 2 minutes usually prevented or delayed mold growth until after the embryo had emerged from the seed coat. In most cases, however, germination occurred so quickly that

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surface sterilization was not necessary, particularly if the seed coat had been adequately broken.

RESULTS - Germination of <u>Sidalcea nelsoniana</u> seeds occurred readily once the seed coat was ruptured and part of it removed. The percentage of germination varied greatly among samples, ranging from 0 to 100 percent (Table 1). Sample numbers refer to different collection localities or collection dates of <u>Sidalcea nelsoniana</u> seed. The lower germination percentages primarily reflect the process of determining that only fully mature seeds will germinate and learning to differentiate between mature and immature seeds. Seeds which were inadequately scarified also show no signs of germination. When these two problems were identified, the germination percentage greatly increased.

Scarified seeds had approximately the same germination percentage regardless of whether they were kept in the light or dark (Table 2). Both groups germinated readily and in the same period of time. The only difference noted was that the cotyledons of seeds kept in the dark did not turn green until the embryos were transplanted into pots and exposed to light.

Seeds with intact seed coats had approximately the same germination percentage regardless of whether they were kept in the dark or in the light (Table 2). Of the 30 seeds kept in the light 4 seeds germinated for a germination percentage of 13 percent; none of the unscarified seeds kept in the dark germinated. The unscarified seeds were kept on moist filter paper for 4 months.

DISCUSSION - Successful laboratory germination of <u>Sidalcea</u> <u>nelsoniana</u> seeds depends on several factors. Only fully mature seeds germinate readily. If immature seeds are scarified some will germinate but most will not. It is most effective to remove a large piece of seed coat. Although puncturing the seed coat with the tip of a probe allows water and oxygen to enter and germination to begin, the embryo is unable to expand and crack open the seed coat and eventually the embryo dies.

The major problem in seedling (embryo) survival was the persistence of the seed coat. The best method for scarification was found to be removal of a piece of the seed coat from the middle of the dorsal surface. If the seed coat was removed from the broad end (cotyledon end) of the seed, the cotyledons would readily emerge but the radicle would not. Removal of the seed coat from the narrow end (radicle end) allowed the radicle to escape but not the cotyledons. When either situation occurred, the seed coat had to be manually removed, with a concomitant greater risk of damaging the embryo. On the other hand, if the embryo was squeezed out of the seed coat with forceps, as previously described, little or no visible damage to the embryo could be detected.

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SAMPLE NUMBER	SEEDS SCARIFIED	SEEDS GERMINATED	PERCENTAGE GERMINATION
1	39	0	0 <sup>a</sup>
2	389	93	24 <sup>a</sup>
3	150	69	46 0b
4	34	3	9 <sup>b</sup> 3 <sup>b</sup>
5	30	1	3
6	106	14	13 <sup>a</sup> 0 <sup>c</sup>
7	22	0	0
8	54	30	56
9	19	13	68
10	9	0	0
11	204	80	0 <sup>a</sup> 39 <sup>a</sup> 0 <sup>a</sup>
12	77	0	04
13	124	60	48
14	79	68	40 86 34 <sup>b</sup>
15	56	19	34
16	77	63	82
17	177	123	69
18	69	58	84
19	32	7	22 <sup>c</sup>
20	29	18	62
21	103	103	100
22	79	64	81
23	81	79	98
24	39	24	62
25	20	11	53
26	18	10	56
27	14	6	43
28	67	54	80
29	36	29	80
30	201	193	96
31	215	205	96 92
32	387	356	
33	87	87	100
TOTALS	3123	1940	62

TABLE 1. Seed Germination in Sidalcea nelsoniana

a. Seeds immature.

b. Seeds inadequately scarified.

c. Seeds insect damaged.

If seed coats still covered the radicle or the cotyledons when embryos were planted in the peat pots, they usually died. When the seed coat covered the cotyledons, the root grew down into the soil but the cotyledons never managed to crack open the seed coat and escape. The epicotyl often lengthened, elevating the seed-enclosed

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SEED TREATMENTS		NUMBER of SEEDS	SEEDS GERMINATED	PERCENTAGE GERMINATION
SEED COAT BROKEN	LIGHT	30	30 <sup>a</sup>	100
	DARK	30	29 <sup>a</sup>	97
SEED COAT INTACT	LIGHT	30	4 <sup>b</sup>	13
	DARK	30	0	0

TABLE 2. Seed Treatments of Sidalcea nelsoniana

a. Germination occurred within 7 days.

b. Germination occurred after 30 days.

cotyledons. If the cotyledons were free of the seed coat but the radicle was not, the cotyledons turned green and started to develop normally; however, the root was unable to shed the seed coat and it remained firmly attached to the root tip. In these cases, the root frequently twisted and appeared to be malformed. Often the root tip was immobilized by the adhering seed coat, which caused the seedling to push itself out of the soil.

Frequently, either the cotyledons or the radicle were physically damaged by the probe during the removal of a piece of the seed coat. If the damage was not too severe, the embryo developed normally. If only one cotyledon was damaged, the other developed sufficiently for the plant's survival. The embryo died if both cotyledons were damaged. If the radicle was damaged, a new root developed, provided that the cotyledons were green and the embryo was left in the petri dish for several days.

The results of these germination studies has implications as to how <u>Sidalcea nelsoniana</u> seeds germinate in nature. As noted above, the seed coat is quite hard. Under natural conditions, this hardness would appear to be the critical impediment to the seed's ability to germinate and develop successfully. If this species' seeds are ruptured in a way that allows water and oxygen to enter but are not broken enough to allow the embryo to expand (as occurred frequently under laboratory conditions), they would be expected to eventually die. Even if the hard seed coat is broken open enough to allow either the cotyledons or the radicle to escape, that also would be insufficient for continued development. It appears that, under natural conditions, either most of the seed coat must be sufficiently

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softened to allow water and oxygen to enter and to allow the embryo to expand and break out of the seed coat, or the seed coat must be broken open completely to allow the entire embryo to escape its confines.

CONCLUSIONS - Mature seeds of <u>Sidalcea</u> <u>nelsoniana</u> germinated readily, with high germination percentages obtained under laboratory conditions, as long as the seeds were first adequately scarified. The seeds need no pre-treatment, other than scarification, to germinate; germination is not affected by light or dark.

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